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Paired associative transcranial alternating current stimulation increases the excitability of corticospinal projections in humans

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Key points

- Repeatedly pairing short trains of peripheral afferent stimulation with bursts (500–1000 ms) of high frequency (≥ 80 Hz) transcranial alternating current stimulation (tACS) over the contralateral primary motor cortex (M1) induces reliable elevations in corticospinal excitability.
- The effect can be obtained using a range of tACS current intensities and frequencies, and with different forms of peripheral afferent stimulation.
- The generation of a temporally discrete cortical event is not a critical determinant of the increases in corticospinal excitability induced by associative stimulation protocols.

Abstract Many types of non-invasive brain stimulation alter corticospinal excitability (CSE). Paired associative stimulation (PAS) has attracted particular attention as its effects ostensibly adhere to Hebbian principles of neural plasticity. In prototypical form, a single electrical stimulus is directed to a peripheral nerve in close temporal contiguity with transcranial magnetic stimulation delivered to the contralateral primary motor cortex (M1). Repeated pairing of the two discrete stimulus events (i.e. association) over an extended period either increases or decreases the excitability of corticospinal projections from M1, contingent on the interstimulus interval. We studied a novel form of associative stimulation, consisting of brief trains of peripheral afferent stimulation paired with short bursts of high frequency (≥ 80 Hz) transcranial alternating current stimulation (tACS) over contralateral M1. Elevations in the excitability of corticospinal projections to the forearm were observed for a range of tACS frequency (80, 140 and 250 Hz), current (1, 2 and 3 mA) and duration (500 and 1000 ms) parameters. The effects were at least as reliable as those brought about by PAS or transcranial direct current stimulation. When paired with tACS, muscle tendon vibration also induced elevations of CSE. No such changes were brought about by the tACS or peripheral afferent stimulation alone. In demonstrating that associative effects are expressed when the timing of the peripheral and cortical events is not precisely circumscribed, these findings suggest that multiple cellular pathways may contribute to a long term potentiation-type response. Their relative contributions will differ depending on the nature of the induction protocol that is used.

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Abbreviations AURC, area under the recruitment curve; CSE, corticospinal excitability; ECR, extensor carpi radialis; EMG, electromyogram; FCR, flexor carpi radialis; FDI, first dorsal interosseous; ISI, interstimulus interval; LTD, long term depression; LTP, long term potentiation; M1, primary motor cortex; MEP, motor evoked potential; NIBS, non-invasive brain stimulation; PAS, paired associative stimulation; PATACS, paired associative transcranial alternating current stimulation; PNS, peripheral nerve stimulation; RMT, resting motor threshold; STDP, spike timing-dependent plasticity; tACS, transcranial alternating current stimulation; tDCS, transcranial direct current stimulation; TMS, transcranial magnetic stimulation; VIBTACS, vibration paired transcranial alternating current stimulation.

Introduction

Among the various forms of non-invasive brain stimulation that have been explored experimentally during the past three decades, paired associative stimulation (PAS) has attracted particular attention as a means by which to investigate the expression of neural plasticity at a systems level in humans (e.g. Müller-Dahlhaus *et al.* 2010). In the prototypical variant (Stefan *et al.* 2000), a single electrical stimulus is applied over a peripheral nerve in advance of transcranial magnetic stimulation (TMS) delivered to the contralateral primary motor cortex (M1). Repeated pairing of the stimuli (i.e. association) over an extended period (e.g. 13–30 min) tends to increase or decrease the excitability of corticospinal projections from M1 in a manner that depends on the interstimulus interval (ISI). If the ISI is set such that the first component of the ascending afferent volley (initiated by the shock to the nerve) reaches the cortex marginally in advance of the magnetic stimulus, 'long term potentiation (LTP)-like' increases in corticospinal excitability (CSE) are observed (Stefan *et al.* 2000). Conversely, if the ISI is adjusted to ensure that the first corollary of the afferent volley registered at M1 arrives following the TMS, 'long term depression (LTD)-like' decreases in corticospinal excitability may be obtained (Wolters *et al.* 2003).

In light of these observations, it has been noted that the effects of PAS are in accordance with Hebbian principles (Stefan *et al.* 2000, 2004; Quartarone *et al.* 2003). More specifically, as the polarity of the induced changes in CSE appears contingent upon the order of the stimulus-generated cortical events, and the effective ISIs lie within a restricted (milliseconds) range, it has been proposed that the resemblance is to spike timing-dependent plasticity (STDP) (Müller-Dahlhaus *et al.* 2010).

Although it is widely assumed that TMS over M1 exerts an effect on chains of interneurons with fixed temporal characteristics that produce a periodic bombardment of corticospinal neurons (Amassian *et al.* 1987), it is also apparent that the magnetic pulse produces complex spreading patterns of cortical activity that are not localised in either space or time. Similarly, the administration of a single shock to a peripheral nerve gives rise to an unfolding series of neural events that can be registered in many parts of the brain. There also exist multiple routes through which the sequelae of TMS applied to M1, and peripheral nerve stimulation, may converge and interact (Carson & Kennedy, 2013). As such, an *a priori* assumption that there is discrete temporal convergence of activity generated by the two associated sources of stimulation is not necessarily warranted. We reasoned that if the generation of a temporally discrete cortical event is not a critical determinant of the effects induced by PAS, it should be possible to replace the TMS element of the induction

protocol with another cortical stimulation modality that is, by design, extended in time.

Transcranial alternating current stimulation (tACS) is one of many other non-invasive methods that can alter brain activity. Moliadze *et al.* (2010) applied high frequency (140 Hz) tACS over M1 for 10 min, and observed consequent increases in the amplitude of motor-evoked potentials (MEPs) elicited by TMS. This effect (which was not obtained for 80 or 250 Hz stimulation) was attributed to an influence of the tACS on endogenous 'ripple range' activity. Sharp wave ripple complexes occur in short (200–700 ms duration) bursts (O'Keefe and Nadel, 1978; Buzsaki *et al.* 1983; Ego-Stengel and Wilson, 2009), and have been associated with the consolidation of some forms of memory (Buzsaki, 2006; Logothetis *et al.* 2012).

In the present study, we used brief bursts of high frequency (≥ 80 Hz) tACS in place of TMS in the context of an associative stimulation protocol. We hypothesised that pairing peripheral afferent stimulation with the application of high frequency alternating current over M1 would lead to changes in corticospinal excitability comparable to those obtained using conventional PAS.

Methods

Participants

Ninety healthy volunteers each participated in one of seven experiments. Their characteristics (with respect to age and sex) are provided below in the description of each experiment. In no instance was there a statistically reliable imbalance in terms of the age or sex of the participants. No individual was involved in more than one experiment. All were right handed according to the Edinburgh handedness inventory (Oldfield, 1971), and gave informed consent to procedures approved by the relevant Queen's University Belfast and Trinity College Dublin Ethics Committees, which were conducted in accordance with the Declaration of Helsinki. For any given experiment, the order of allocation to conditions was pseudo-randomised and counterbalanced across participants. In line with current recommendations (Nitsche *et al.* 2008), successive testing sessions were separated by at least 7 days.

Recording procedures

The participants were seated with the upper limbs supported and stabilized by vacuum cushions, the forearms in mid-pronation and the elbows semi-flexed (100–120 deg). Electromyographic (EMG) activity was recorded from the right flexor carpi radialis (FCR) and extensor carpi radialis brevis (ECR) muscles, using pairs of silver chloride (AgCl) electrodes. EMG signals

were amplified (gain = 1000), bandpass filtered (20 or 30–1000 Hz) and digitized at a sampling rate of 4 kHz.

Magnetic stimuli were delivered to the left primary motor cortex (M1) by a Magstim 200 stimulator using a figure of eight coil (internal wing diameter 70 mm), located at the optimal position ('hot spot') to obtain a motor-evoked potential (MEP) in the FCR muscle of the contralateral (right) arm. The coil was placed so that the axis of intersection between the two loops was orientated at approximately 45 deg to the sagittal plane, to induce posterior to anterior current flow across the motor strip. Once the hot spot was established, the lowest stimulation intensity at which MEPs with peak-to-peak amplitude of approximately 50 μ V were evoked in at least 5 of 10 consecutive trials was taken as resting motor threshold (RMT).

Prior to each intervention (Pre), an MEP recruitment curve was obtained by delivering TMS at 10% increments of intensity between 90 and 160% of the RMT. Six stimuli were delivered at each level of intensity. A further 12 stimuli were delivered at 120% RMT. The order of delivery was randomised. The interval between successive stimuli varied between 4 and 6 s. The total duration of the sequence was approximately 5 min. The average MEP amplitudes obtained at 90 and 100% RMT were calculated to ensure that the threshold had been correctly determined. In cases where the averaged MEPs for these intensities did not correspond to the expected values (i.e. <50 μ V at 90% RMT, 50–100 μ V at 100% RMT), the threshold intensity was adjusted accordingly and another recruitment curve was obtained. Equivalent sets of stimuli (without adjustments of threshold) were delivered immediately following the intervention (Post0) and at 10 (Post10), 20 (Post20) and 30 (Post30) minutes thereafter. The first of these sets (i.e. Post0) always commenced within 30 s following completion of the intervention. There was a break of 5 min after the delivery of each such set of stimuli prior to commencement of the subsequent set.

Methods used in the interventions

Peripheral nerve stimulation (PNS). A constant-current stimulator (Grass S88 Dual Output Square Pulse Stimulator; Grass Technologies, West Warwick, RI, USA) was used to locate the motor point of FCR by moving a bipolar surface electrode over the muscle belly. The principal identification criterion was a reliable, visible displacement of the FCR tendon at the wrist. Two AgCl electrodes were affixed in line with the orientation of the muscle fibres – one on either side of the location thus defined. These were used for both stimulation and EMG recording. The intensity of stimulation delivered during an intervention was the minimum at which visible displacement of the FCR tendon was observed. Depending

on experimental condition, 10 Hz trains of 3, 5 or 10 pulses (each of 1 ms duration) were employed.

tACS. Flexible electrode paddles were placed within two saline-soaked 5 cm \times 5 cm sponges and fixed securely on the scalp using non-conducting elastic straps. One electrode was placed over left M1 at the FCR 'hot spot' determined previously by TMS. The other electrode was placed over the contralateral supraorbital area. A battery-driven stimulator (A-M Systems Model 2200, Carlsborg, WA, USA) controlled by Signal software (Cambridge Electronic Design, Cambridge, UK) was used to deliver short (≤ 1000 ms) bursts of bipolar sinusoidal alternating current at a fixed frequency and amplitude (values dependent on experimental condition). The current density was 0.04 mA cm⁻² at 1 mA, 0.08 mA cm⁻² at 2 mA and 0.12 mA cm⁻² at 3 mA. Electrode impedance was monitored and maintained below 5 k Ω . In all tACS conditions, 180 bursts were delivered at approximately 10 s intervals, corresponding to a stimulation period of 30 min.

Transcranial direct current stimulation (tDCS) and sham stimulation. The anode was placed over left M1 at the FCR 'hot spot' determined previously by TMS. The cathode was placed over the contralateral supraorbital area. In the tDCS condition, the stimulator was driven by Spike software (Cambridge Electronic Design) to deliver current at 1 mA for 30 min, including a 10 s period at the beginning and at the end when the current was ramped up/down. For the sham stimulation condition, to provide a skin tingling sensation, the current was ramped up to 1 mA over the first 10 s and ramped back down to zero over the subsequent 10 s.

Transcranial magnetic stimulation (PAS condition). We utilised a variant of PAS described by Castel-Lacanal *et al.* (2007) (see also Carson *et al.* 2013). The peripheral nerve stimulation consisted of a train of five pulses delivered at 10 Hz. Single pulse TMS at 120% RMT (FCR) was delivered 25 ms after the final pulse of the train. The protocol was in other respects equivalent to that described for tACS.

Muscle vibration. Vibration was applied to the distal tendon of the right FCR, 3 cm proximal to the radiocarpal joint, by means of an exciter (Type 4810 mini-shaker, Bruel & Kjaer, Sydney, Australia) driven by Signal software (Cambridge Electronic Design) via a power amplifier (Ling Dynamic Systems UK model PA 25, Royston, UK). A notched plastic probe attached to the exciter was applied perpendicular to the tendon with a comfortable but firm load, which remained constant throughout the experiment through stable fixation of both the arm and the exciter. Sinusoidal vibration at 80 Hz was delivered

at the threshold level necessary to evoke the kinaesthetic illusory sensation (Naito *et al.* 1999). To define this threshold participants were asked to look away from the hand while the amplitude of the vibration was increased gradually over approximately 20 s. The participants were asked to report the first moment at which they perceived the wrist to flex towards the body midline. The threshold was defined as the mean amplitude of vibration necessary to evoke the illusion in three such trials.

General procedures

The procedures for the seven experiments differed only in the parameters of stimulation applied during the intervention. Figure 1 illustrates the time course of the experiments. In all experiments, regardless of intervention condition, PNS was used to establish the motor point and define a threshold stimulation intensity.

The first MEP recruitment curve was recorded prior to commencement of the intervention. Thereafter, the tACS electrodes were placed on the scalp, and the bipolar surface electrodes on the FCR were switched from EMG measurement to PNS delivery, regardless of whether the forthcoming condition required peripheral stimulation. Following the (30 min duration) intervention, further recruitment curves were obtained at fixed intervals ($\sim 0, 10, 20$ and 30 min).

In five of the seven experiments the participants were asked to attend testing on three occasions. These were separated by at least 1 week. Testing was conducted at the same time of day for each participant to control for potential effects of circadian cortisol fluctuations (Sale *et al.* 2007). In examining the effects of sham stimulation (Experiment 4) and peripheral nerve stimulation alone (Experiment 7) – see below – there were one and two sessions, respectively. Testing was carried out double-blinded in experiments 1, 2, 3 and 7, and in all other experiments the participants remained naïve to the specific parameters of stimulation. In all cases, a jitter (± 5000 ms maximum) was introduced in relation to the timing of successive stimulation events (mean separation 10 s) to ensure that, for conditions in which at least one modality was perceptible (tACS and tDCS were in general not perceived), the participants could not anticipate their onset.

Interventions

Experiment 1 – paired associative transcranial alternating current stimulation (PATACS) with variations of tACS frequency. Each of the 180 paired stimulation events (separated by ≈ 10 s) comprised a train of PNS (five pulses at 10 Hz). The train commenced 25 ms prior to the onset of a 500 ms duration burst of 2 mA tACS. In the three

separate conditions, the frequency of tACS was 80, 140 or 250 Hz (Fig. 2A). The 12 participants (6 male) were aged 18–28 years (mean, 21.8 ± 4.3 years).

Experiment 2 – PATACS with variations of tACS current. Each of the 180 paired stimulation events comprised a train of PNS (five pulses at 10 Hz). The train commenced 25 ms prior to the onset of a 500 ms duration burst of 140 Hz tACS. In three separate conditions, the tACS current was either 1, 2 or 3 mA (Fig. 2B). The 12 participants (3 male) were aged 20–29 years (mean, 24.0 ± 3.7 years).

Experiment 3 – PATACS with variations of tACS and PNS duration. Each of the 180 paired stimulation events comprised a train of PNS at 10 Hz which commenced 25 ms prior to the onset of a burst of 140 Hz 2 mA tACS. In the first condition PNS (train of three pulses) was paired with tACS lasting 250 ms. In the second, PNS (five pulses) was paired with tACS of duration 500 ms. In the third condition, PNS (10 pulses) was paired with tACS of duration 1000 ms (Fig. 2C). The 12 participants (3 male) were aged 18–40 years (mean, 24.7 ± 6.2 years).

Experiment 4 – sham stimulation. Transcranial unipolar direct current was ramped up from 0 mA to 1 mA over ten seconds, then ramped back down to 0 mA during the ten seconds following. During the 30 min intervention period peripheral afferent stimulation was not applied (Fig. 2D). The twelve participants (4 male) were aged 20–30 (mean, 22.7 ± 3.3 years).

Experiment 5 – PATACS with variation of peripheral afferent stimulation modality. In the vibration paired TACS (VIBTACS) condition, 80 Hz vibration of the FCR muscle tendon was applied for 500 ms at the kinaesthetic illusory threshold. This commenced 25 ms prior to the onset of a 500 ms burst of 140 Hz 2 mA tACS. This pairing was repeated approximately every 10 s for 30 min. In the PATACS condition, each of the 180 paired stimulation events comprised a train of PNS (five pulses at 10 Hz), which commenced 25 ms prior to the onset of a burst of 140 Hz 2 mA tACS. In a further control condition, 500 ms bursts of 140 Hz 2 mA tACS were delivered at approximately 10 s intervals for 30 min. In this condition there was no peripheral stimulation (Fig. 2E). The 12 participants (5 male) were aged 18–24 years (mean, 20.2 ± 2.0 years).

Experiment 6 – comparison of PATACS with PAS and tDCS. In the PATACS condition, each of the 180 paired stimulation events comprised a train of PNS (five pulses at 10 Hz), which commenced 25 ms prior to the onset of a burst of 140 Hz 1 mA tACS. In the PAS condition, single pulse TMS at 120% RMT (FCR) was delivered 25 ms after

the final pulse of the PNS train (Castel-Lacanal *et al.* 2007; Carson *et al.* 2013). In the tDCS condition, unipolar direct current was ramped up from 0 to 1 mA over a period of 10 s. Stimulation continued at a constant current of 1 mA for 30 min, before being ramped back down to 0 mA over 10 s (Fig. 2F). The 12 participants (9 male) were aged 20–31 years (mean, 24.6 ± 4.6 years).

Experiment 7 – comparison of PATACS with PNS alone. In the PATACS condition, each of the 180 paired stimulation events comprised a train of PNS (five pulses at 10 Hz), which commenced 25 ms prior to the onset of a burst of 140 Hz 2 mA tACS. In the PNS alone condition, 180 PNS events were delivered over the course of the 30 min intervention period in accordance with the PATACS schedule. These were preceded by three paired PNS–tACS events. Eighteen participants were enrolled in this experiment. One person withdrew owing to dislike of the PNS. With respect to the remaining 17 participants (10 male), they were aged 18–32 years (mean, 24.1 ± 3.8 years).

Data analysis

The root mean square (rms) of the background EMG recorded in FCR and ECR was calculated for a window 93–3 ms before TMS onset. If the value was greater than $5 \mu\text{V}$ for either muscle, the corresponding MEP was disregarded. As a further means of eliminating instances in which elevated excitability of the spinal motoneuron pool may have influenced the MEP amplitude, we first calculated for each participant (separately for FCR and ECR) the quartiles for all background rms EMG values retained following the screening procedure described above. In the event that an individual rms value was above the upper whisker of the distribution (in this instance set to the third quartile plus 1.5 times the interquartile range) the corresponding MEP was disregarded. Overall, 88.4% of the responses were retained.

For the retained recordings, the mean (peak-to-peak) amplitude of the MEPs elicited at the eight respective stimulation intensities was calculated. For each time of measurement (Pre, Post0, Post10, Post20 and Post30), the summated area under the recruitment curve (AURC), bounded by magnetic stimulation intensity and MEP amplitude (in units of mV.T), was obtained using the trapezoidal rule. It has been demonstrated elsewhere (Carson *et al.* 2013) that the AURC is an extremely reliable measure of the state of corticospinal projections to hand and forearm muscles, which has construct, face and concurrent validity.

It is widely recognised (e.g. Abelson, 1995) that in contrast to between-groups analyses, inferential tests in repeated measures designs, including ANOVA and mixed effects models, are highly vulnerable to violation of underlying assumptions, including normality of the sample distribution. To address this issue, the normality of the distribution of AURC values obtained in each analysis cell (i.e. separately for each experiment) was assessed using the Shapiro–Wilks test. On the basis of these analyses it was established that for 42% (38/90) of the cells, the AURC values were not normally distributed ($P < 0.05$). To increase the symmetry of the sample distribution for the purposes of inferential analyses, the AURC values were therefore subject to a log transformation. Following application of the transformation, there were no cells for which the outcome of the Shapiro–Wilks test indicated that the values were not normally distributed.

Mixed effects models in which participant was a random effect, and time was a fixed effect (levels = Pre, Post0, Post10, Post20, Post30), were conducted separately for each experiment (using the lmerTest package in R). The fitting of the models employed restricted maximum likelihood (REML) estimation and an unstructured covariance matrix. On the basis of these models, planned contrasts were conducted between the (log) AURC value obtained prior to the intervention (Pre), and the (log) AURC calculated for each time point following the intervention (Post0, Post10, Post20, Post30). The exact

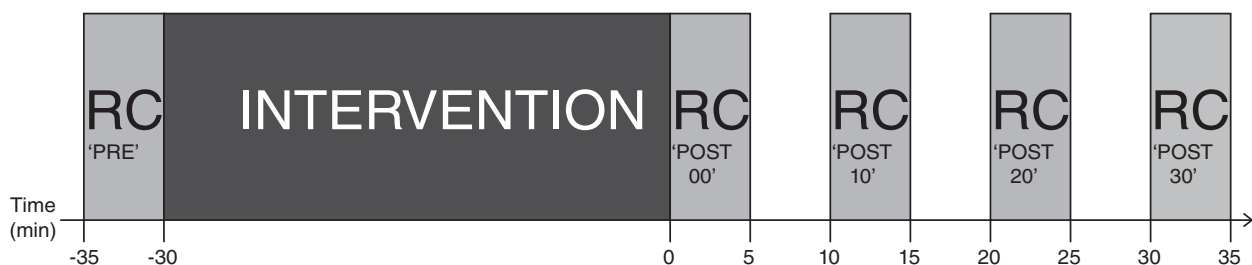


Figure 1. Time course of each experimental session

A recruitment curve ('Pre') was measured at the beginning of testing followed by a 30 min intervention that differed according to the experimental condition. Further recruitment curves were measured immediately following ('Post00') and at 10 min ('Post10'), 20 min ('Post20') and 30 min ('Post30') following the completion of the intervention.

probabilities associated with each comparison are reported in Tables 1–7. The relevant degrees of freedom were obtained using Kenward–Roger’s approximation that, in the case of the balanced designs employed in the present study, yields values equivalent to those of a repeated measures ANOVA design.

A series of supplementary analyses (using mixed effects models) were performed that contrasted at each post-intervention time point the (log) AURC values obtained in the various conditions employed in each study. In all such instances, the AURC value obtained prior to the intervention in each condition was used as a covariate.

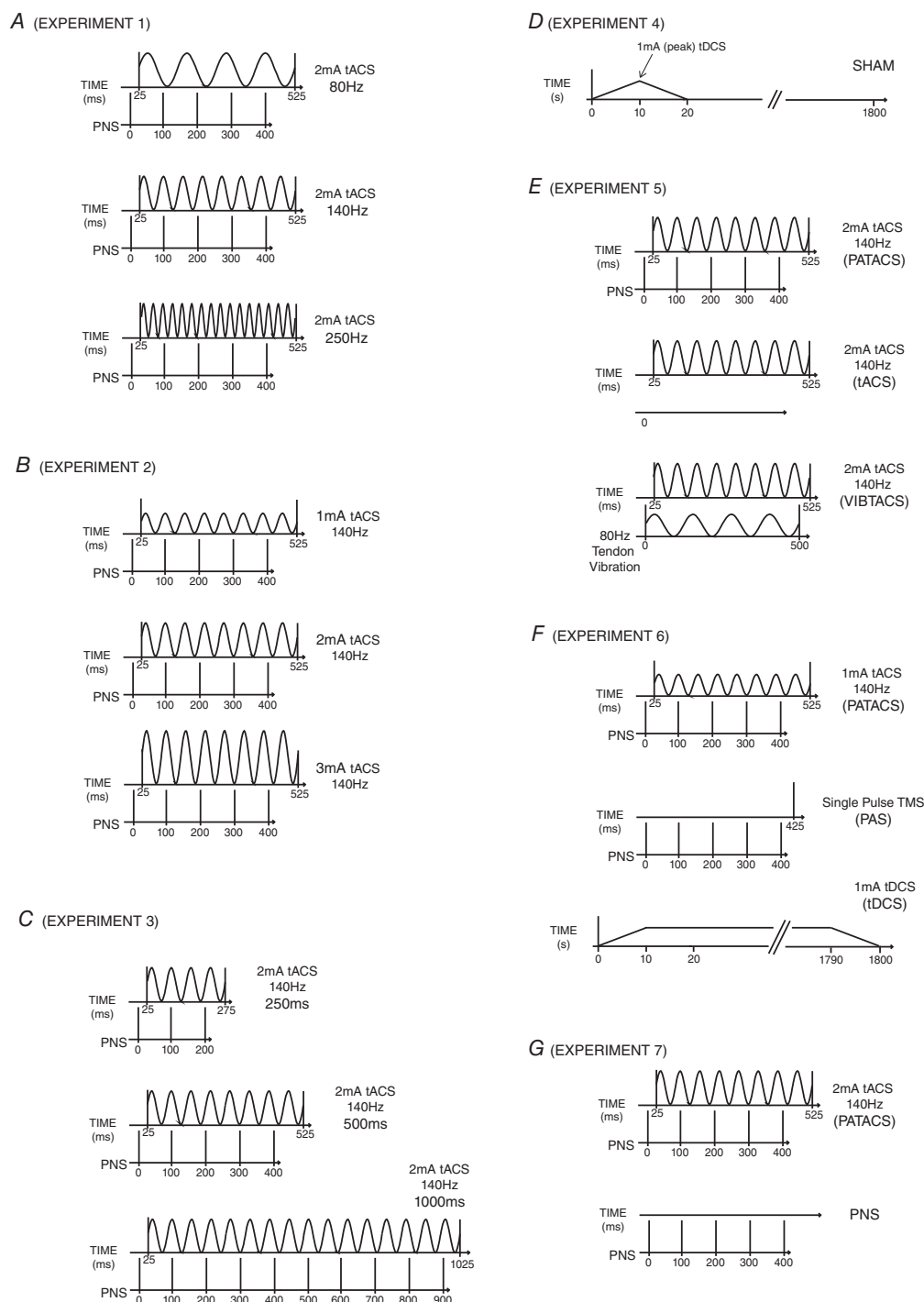


Figure 2. Stimuli presented during the interventions applied in each experiment

In its prototypical form, PATACS comprised a train of electrical peripheral nerve stimulation (PNS – five pulses at 10 Hz) commencing 25 ms prior to the onset of a 500 ms duration burst of tACS with frequency 140 Hz and

Table 1. Experiment 1: *F* ratios, *P* values and effect sizes for comparisons between the AURC obtained prior to the intervention (Pre), and the values obtained at each of four time points following the intervention

Condition	Pre vs.	<i>F</i> _{1,88}	<i>P</i> value	Effect size (<i>f</i>)
80 Hz	Post00	5.59	0.020	0.24
	Post10	6.36	0.010	0.26
	Post20	8.67	0.004	0.30
	Post30	17.78	<0.001	0.44
140 Hz	Post00	0.14	0.706	0.04
	Post10	3.10	0.081	0.18
	Post20	2.28	0.135	0.16
	Post30	24.40	< 0.001	0.51
250 Hz	Post00	0.97	0.330	0.10
	Post10	1.37	0.250	0.12
	Post20	3.77	0.056	0.20
	Post30	12.75	< 0.001	0.37

Table 2. Experiment 2: *F* ratios, *P* values and effect sizes for comparisons between the AURC obtained prior to the intervention (Pre) and the values obtained at each of four time points following the intervention

Condition	Pre vs.	<i>F</i> _{1,88}	<i>P</i> value	Effect size (<i>f</i>)
1 mA	Post00	1.41	0.230	0.12
	Post10	4.96	0.029	0.23
	Post20	4.77	0.032	0.23
	Post30	14.59	<0.001	0.40
2 mA	Post00	0.18	0.670	0.04
	Post10	1.36	0.250	0.12
	Post20	4.81	0.031	0.23
	Post30	5.60	0.020	0.25
3 mA	Post00	0.97	0.330	0.10
	Post10	2.22	0.140	0.15
	Post20	8.19	0.005	0.30
	Post30	11.74	< 0.001	0.36

To further assist in the interpretation of the tests of significance, in particular with a view to comparing the respective conditions included in each experiment, the unbiased effect size index for ANOVA (*f*) (Cohen, 1988) was calculated for each planned contrast following Nakagawa and Cuthill (2007). This is a dimensionless index, which describes the degree of departure from no effect, in other words the degree to which the phenomenon is manifested. A small effect size is considered by convention to be indicated by an *f* of 0.1, a medium effect size by an *f* of 0.25 and a large effect size by an *f* of 0.4.

Results

Experiment 1 – PATACS with variations of tACS frequency

In the 80 Hz condition, indices of corticospinal excitability (AURC) were elevated reliably (relative to initial values) at all time points following the intervention (Fig. 3). The largest increase was observed 30 min following the cessation of stimulation. A statistically reliable increase was observed in the 140 Hz condition at 30 min

after intervention. In the 250 Hz condition, AURC values increased monotonically following the cessation of stimulation. They were elevated reliably relative to initial values at 30 min after intervention (Table 1). The AURC values obtained in the three conditions were not, however, distinguished reliably from each other ($F_{1,65.4}$, $P = 0.05$ – 0.93 , $f = 0.01$ – 0.23).

Experiment 2 – PATACS with variations of tACS current

When 1 mA tACS current was employed, elevations in CSE following the interventions were observed 10 min following the cessation of paired stimulation. These increased in magnitude thereafter (Fig. 4). In the 2 mA condition, a similar trend expressed at Post20 (Table 2) was expressed reliably 30 min following the cessation of paired stimulation. In the 3 mA condition, elevations of CSE were evident 20 and 30 min following paired stimulation. The AURC values obtained in the three conditions were not, however, distinguished reliably from each other ($F_{1,64.6}$, $P = 0.42$ – 0.83 , $f = 0.01$ – 0.09).

current 2 mA. There were 180 such paired stimuli, one every ~10 s over a period of 30 min. A, in Experiment 1 the frequency of tACS (80, 140 or 250 Hz) was varied across conditions. B, in Experiment 2 the current intensity (1, 2 and 3 mA) was varied. C, in Experiment 3 the duration of stimulation was varied – either a train of three PNS pulses was paired with 250 ms of tACS, five PNS pulses were paired with 500 ms of tACS, or 10 PNS pulses were paired with 1000 ms tACS. D, in Experiment 4 transcranial unipolar direct current was ramped up from 0 to 1 mA over 10 s, then ramped back down to 0 mA during the 10 s following. E, in Experiment 5 the PATACS condition consisted of the prototypical form of stimulation. The tACS condition involved AC stimulation unaccompanied by PNS. In the VIBTACS condition 80 Hz vibration of the muscle tendon was applied for 500 ms, commencing 25 ms prior to the onset of 500 ms tACS. F, in Experiment 6, PATACS was delivered in its prototypical form, with the exception that the tACS current was 1 mA. In the PAS condition, single pulse TMS at 120% RMT (FCR) was delivered 25 ms after the final pulse of the PNS train. In the tDCS condition, 1 mA ‘anodal’ direct current was applied for 30 min. G, in Experiment 7, PATACS was delivered in its prototypical form. The PNS condition involved electrical peripheral afferent stimulation unaccompanied by tACS.

Table 3. Experiment 3: *F* ratios, *P* values and effect sizes for comparisons between the AURC obtained prior to the intervention (Pre) and the values obtained at each of four time points following the intervention

Condition	Pre vs.	$F_{1,88}$	<i>P</i> value	Effect size (<i>f</i>)
250 ms	Post00	1.14	0.290	0.11
	Post10	1.14	0.290	0.11
	Post20	0.68	0.410	0.08
	Post30	2.34	0.130	0.16
500 ms	Post00	0.83	0.363	0.09
	Post10	1.31	0.255	0.12
	Post20	0.41	0.524	0.07
	Post30	1.56	0.214	0.13
1000 ms	Post00	0.16	0.694	0.04
	Post10	1.69	0.198	0.13
	Post20	5.96	0.017	0.25
	Post30	4.34	0.040	0.22

Table 4. Experiment 4: *F* ratios, *P* values and effect sizes for comparisons between the AURC obtained prior to the intervention (Pre) and the values obtained at each of four time points following the intervention

Condition	Pre vs.	$F_{1,44}$	<i>P</i> value	Effect size (<i>f</i>)
Sham	Post00	0.36	0.554	0.09
	Post10	3.28	0.077	0.26
	Post20	0.03	0.861	0.03
	Post30	0.09	0.763	0.04

larger than those recorded prior to the intervention, these changes were not statistically reliable (Table 3). When the duration of the stimulation events was 1000 ms, CSE was elevated reliably at 20 and 30 min after paired stimulation (Fig. 5). The AURC values obtained in the three conditions were not, however, distinguished reliably from each other ($F_{1,64.7}$, $P = 0.19$ – 0.98 , $f = 0.003$ – 0.16).

Experiment 3 – PATACS with variations of tACS and PNS duration

Although the mean AURC values obtained following paired stimulation in the 250 and 500 ms conditions were

Experiment 4 – sham stimulation

Sham stimulation failed to produce reliable changes in corticospinal excitability (Fig. 6 and Table 4).

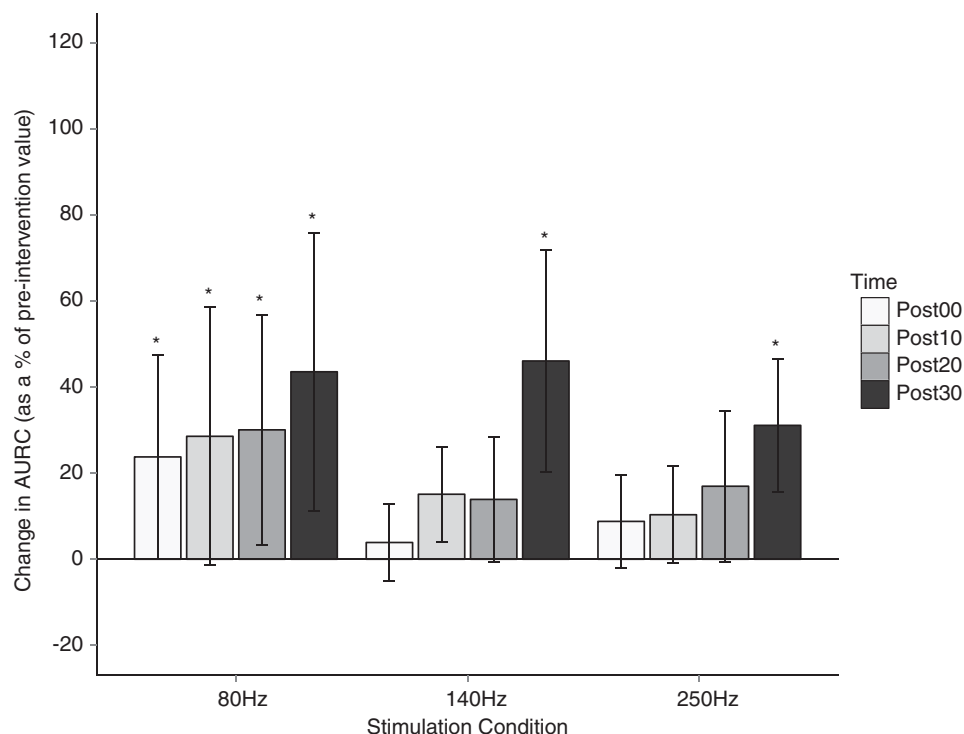


Figure 3. For Experiment 1, in which the frequency of tACS was varied, the AURC for each post-intervention time point is expressed as the (percentage) change relative to the value obtained prior to the intervention

All values are the mean of 12 participants. The error bars are the corresponding 95% confidence intervals calculated across participants. Values recorded following the intervention that differed reliably ($P < 0.05$) from those obtained prior to the intervention are represented by an asterisk symbol above the error bar.

Table 5. Experiment 5: *F* ratios, *P* values and effect sizes for comparisons between the AURC obtained prior to the intervention (Pre) and the values obtained at each of four time points following the intervention

Condition	Pre vs.	$F_{1,88}$	<i>P</i> value	Effect size (<i>f</i>)
PATACS	Post00	1.52	0.221	0.13
	Post10	1.83	0.180	0.14
	Post20	2.11	0.150	0.15
	Post30	11.20	0.001	0.36
TACS	Post00	0.09	0.767	0.03
	Post10	1.03	0.312	0.11
	Post20	0.43	0.514	0.07
	Post30	0.01	0.921	0.01
VIBTACS	Post00	15.43	<0.001	0.41
	Post10	20.75	<0.001	0.48
	Post20	12.28	<0.001	0.37
	Post30	12.10	<0.001	0.37

Table 6. Experiment 6: *F* ratios, *P* values and effect sizes for comparisons between the AURC obtained prior to the intervention (Pre) and the values obtained at each of four time points following the intervention

Condition	Pre vs.	$F_{1,88}$	<i>P</i> value	Effect size (<i>f</i>)
PATACS	Post00	1.25	0.266	0.12
	Post10	2.01	0.160	0.15
	Post20	1.37	0.245	0.12
	Post30	4.16	0.044	0.21
PAS	Post00	1.63	0.204	0.13
	Post10	2.50	0.117	0.16
	Post20	1.25	0.267	0.12
	Post30	2.59	0.111	0.17
TDCS	Post00	0.21	0.650	0.05
	Post10	2.94	0.090	0.18
	Post20	1.80	0.184	0.14
	Post30	2.44	0.122	0.16

Experiment 5 – PATACS with variation of peripheral afferent stimulation modality

In the PATACS condition, reliable elevations in corticospinal excitability were obtained 30 min following the

cessation of paired stimulation (Fig.7). In the VIBTACS condition, when measured at all time points following the cessation of paired (tACS and tendon vibration) stimulation, the AURC was markedly larger than pre-intervention values (Table 5). No reliable increases in

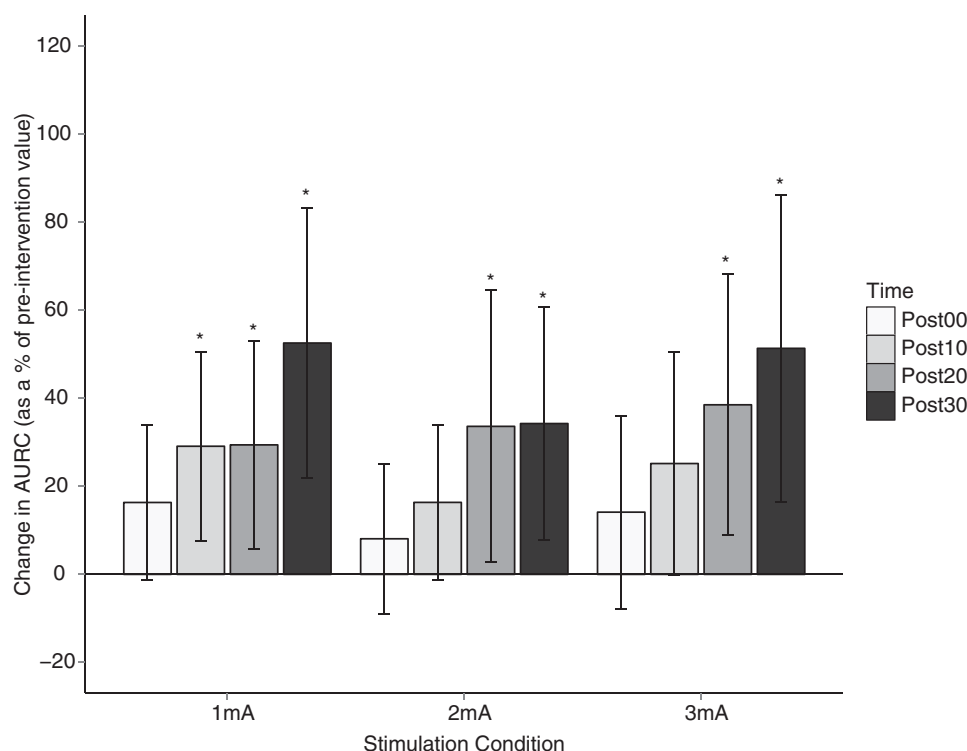


Figure 4. For Experiment 2, in which the tACS current was varied, the AURC for each post-intervention time point is expressed as the (percentage) change relative to the value obtained prior to the intervention

All values are the mean of 12 participants. The error bars are the corresponding 95% confidence intervals calculated across participants. Values recorded following the intervention that differed reliably ($P < 0.05$) from those obtained prior to the intervention are represented by an asterisk above the error bar.

Table 7. Experiment 7: *F* ratios, *P* values and effect sizes for comparisons between the AURC obtained prior to the intervention (Pre) and the values obtained at each of four time points following the intervention

Condition	Pre vs.	$F_{1,64}$	<i>P</i> value	Effect size (<i>f</i>)
PATACS	Post00	3.16	0.074	0.22
	Post10	5.22	0.022	0.29
	Post20	5.12	0.024	0.28
	Post30	3.93	0.046	0.25
PNS	Post00	1.17	0.273	0.14
	Post10	0.06	0.799	0.03
	Post20	0.47	0.484	0.09
	Post30	0.06	0.810	0.03

corticospinal excitability were observed at any time point following the administration of tACS alone (i.e. without peripheral stimulation). Additional analyses revealed that in the PATACS condition, corticospinal excitability was greater than that observed in the tACS only condition, when contrasted (d.f. = 1, 61.8) at 30 min following the end of the intervention ($P = 0.02$, $f = 0.28$). Upon the end of the intervention (Post00), the AURC values obtained in the VIBTACS condition were larger than those in the

tACS only condition ($P = 0.02$, $f = 0.30$), with a similar pattern being expressed 10 min ($P = 0.07$, $f = 0.23$) and 30 min ($P = 0.07$, $f = 0.23$) thereafter. The AURC values obtained in the PATACS and VIBTACS conditions were not distinguished reliably from each other ($F_{1,61.8}$, $P = 0.10$ – 0.69 , $f = 0.05$ – 0.21).

Experiment 6 – comparison of PATACS with PAS and tDCS

In the PATACS condition, reliable elevations in corticospinal excitability were obtained 30 min following the cessation of paired stimulation (Fig. 7). In the tDCS and PAS conditions, no changes in corticospinal excitability met conventional criteria for statistical significance (Table 6). The AURC values obtained in the three conditions were not distinguished reliably from each other ($F_{1,65.2}$, $P = 0.24$ – 0.99 , $f = 0.002$ – 0.14).

Experiment 7 – comparison of PATACS with PNS alone

In the PATACS condition, reliable elevations in corticospinal excitability were obtained 10, 20 and 30 min following the cessation of paired stimulation (Fig. 9). No reliable increases in corticospinal excitability were observed at any time point following the administration

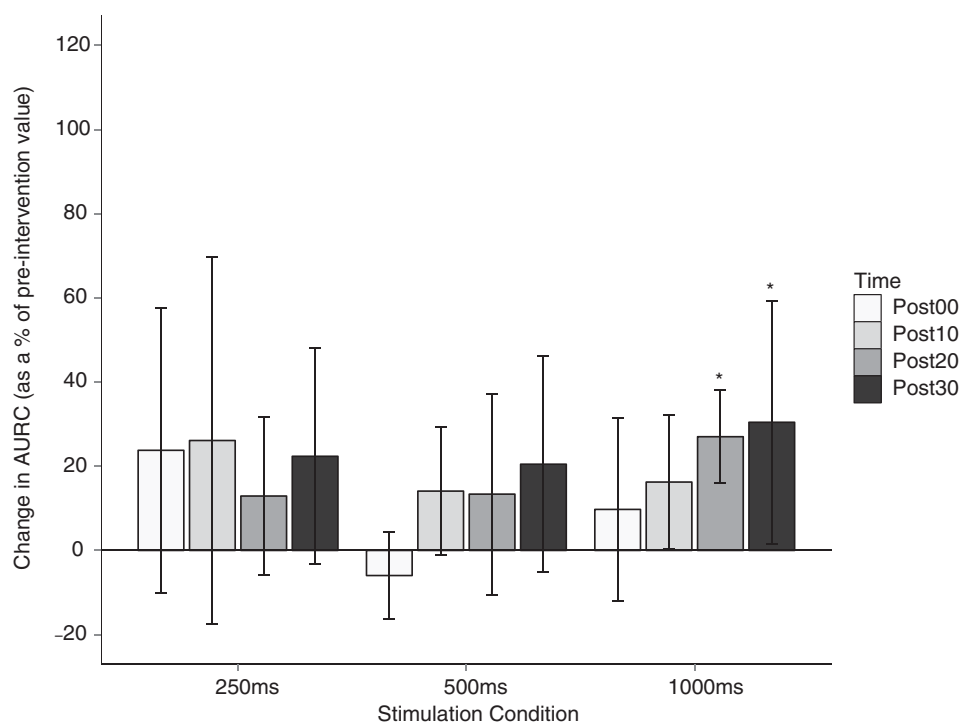


Figure 5. For Experiment 3, in which the duration of the paired stimulation events was varied, the AURC for each post-intervention time point is expressed as the (percentage) change relative to the value obtained prior to the intervention

All values are the mean of 12 participants. The error bars are the corresponding 95% confidence intervals calculated across participants. Values recorded following the intervention that differed reliably ($P < 0.05$) from those obtained prior to the intervention are represented by an asterisk above the error bar.

of PNS alone (i.e. without tACS) (Table 7). Additional analyses revealed that in the PATACS condition, corticospinal excitability was greater than that observed in the PNS only condition, when contrasted (d.f. = 1, 47.7) at 0 min ($P = 0.01$, $f = 0.38$), 10 min ($P = 0.05$, $f = 0.28$) and 30 min ($P = 0.04$, $f = 0.31$) following the end of the intervention.

Pooled data – PATACS comprising 140 Hz, 2 mA tACS of 500 ms duration

Sixty-four participants (drawn from five experiments) were exposed to the same PATACS protocol (i.e. PNS paired with 140 Hz, 2 mA tACS of 500 ms duration). In analysing these pooled data, the relatively large sample size permitted the calculation of confidence intervals (95% CI) for the effect size estimates (Smithson, 2001). When assessed 30 min following the cessation of stimulation, the observed changes in corticospinal excitability constituted a medium to large effect (95% CI (Cohen's f unbiased) 0.27–0.48; $F_{1,251} = 36.5$, $P < 0.0001$). There was a small to medium effect when assessed 10 min (95% CI (Cohen's f unbiased) 0.13–0.34; $F_{1,251} = 14.2$, $P = 0.0002$) and 20 min

(95% CI (Cohen's f unbiased) 0.14–0.35; $F_{1,251} = 15.5$, $P = 0.0001$) after stimulation. There was no basis upon which to conclude that corticospinal excitability was elevated immediately following the intervention (95% CI (Cohen's f unbiased) 0–0.20; $F_{1,251} = 2.27$, $P = 0.13$).

Additional observations

In Experiment 1, 11 of the 12 participants reported experiencing phosphenes in the 80 Hz condition. No such percepts were reported in any of the other conditions or experiments.

Discussion

We have demonstrated that a novel form of associative stimulation, in which bursts of tACS are paired with trains of peripheral afferent stimulation, increases the excitability of corticospinal projections to the forearm. In the context of a series of seven experiments (engaging distinct groups of participants), in which various stimulation parameters were manipulated, it was evident that the defining effect is highly replicable. Most notably,

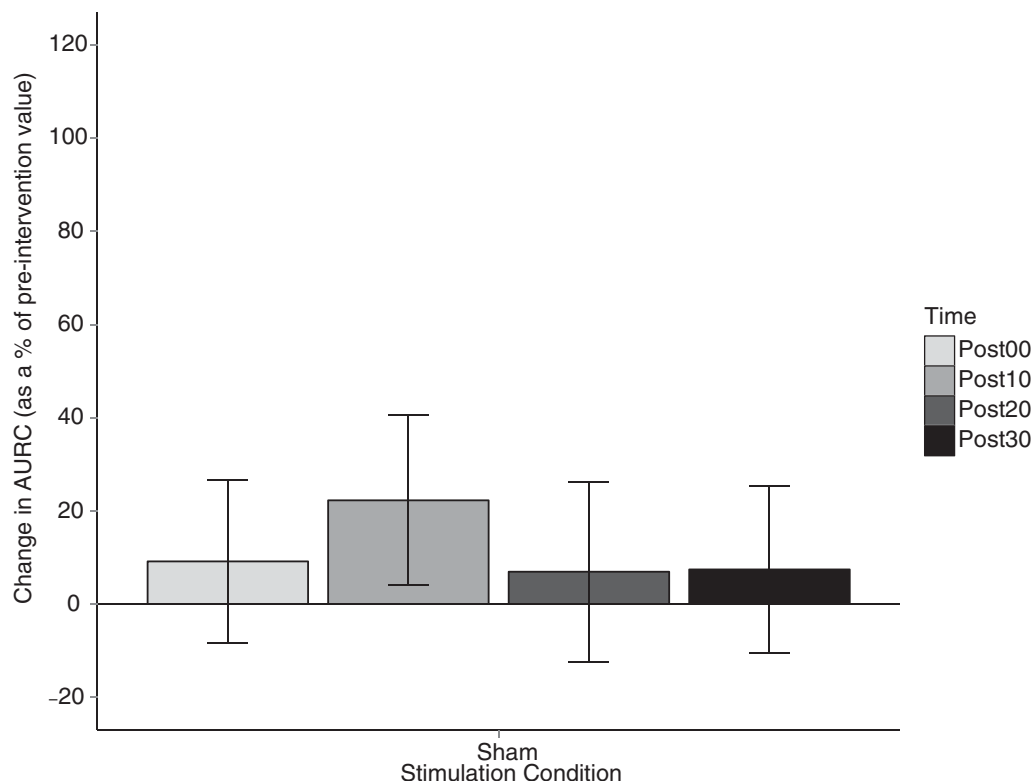


Figure 6. For Experiment 4, in which sham stimulation was delivered, the AURC for each post-intervention time point is expressed as the (percentage) change relative to the value obtained prior to the intervention

All values are the mean of 12 participants. The error bars are the corresponding 95% confidence intervals calculated across participants. There were no instances in which values recorded following the intervention differed reliably from those obtained prior to the intervention.

it is not contingent upon temporally discrete cortical or peripheral stimulation events. Rather it was obtained using extended (500 and 1000 ms) periods of excitation.

Among the many means by which LTP and LTD can be induced in reduced preparations, it has been suggested (e.g. Wolters *et al.* 2005) that STDP occupies a unique position in so much as the polarity of the induced change in synaptic efficacy is determined by the sequence of pre- and postsynaptic neuronal activity (for reviews see Dan & Poo, 2004; Markram, Gerstner & Sjöström, 2011). In the classical model of STDP (e.g. Song *et al.* 2000), strengthening (potentiation) arises if the presynaptic neuron fires no more than 50 ms in advance of the postsynaptic neuron (Feldman, 2000), whereas weakening (depression) occurs if postsynaptic spikes precede presynaptic action potentials (or transpire without activity in the presynaptic neuron) (Levy & Steward, 1983; Bi & Poo 1998; Cooke & Bliss, 2006).

In foundational descriptions of PAS (e.g. Wolters *et al.* 2003) it was highlighted that increases in corticospinal excitability are achieved if PNS is timed such that the initial phase of input to M1 arising as its corollary occurs synchronously with the delivery of a magnetic pulse over that region of cortex. If the relative timing is adjusted

such that TMS is applied prior to the time at which a corollary of the (single pulse) PNS is likely to reach M1, repeated pairings may lead to a subsequent reduction in the excitability of corticospinal projections. As these initial reports indicated not only that the order of the stimulus-generated cortical events is critical, but also that the effective ISIs lie within a very restricted range, it was concluded that PAS-induced adaptation represents a form of associative LTP and LTD which exhibits the defining features of STDP (Müller-Dahlhaus *et al.* 2010).

Increases in corticospinal excitability have been observed previously following the application of associative protocols that comprised extended trains of afferent stimulation. Ridding and Taylor (2001) administered TMS 25 ms after the *onset* of 500 ms trains applied over the motor point of first dorsal interosseus (FDI). Following a 30 min intervention, increases in the amplitude of MEPs elicited in FDI were observed (see also McKay *et al.* 2002). When the TMS is administered 25 ms following the *last* shock of the train, effects of a similar nature are obtained when either the ECR (Castel-Lacanal *et al.* 2007) or the FCR (Carson *et al.* 2013) motor point is in receipt of stimulation. While at first glance these results suggest that the range of effective ISIs is larger than is supposed in

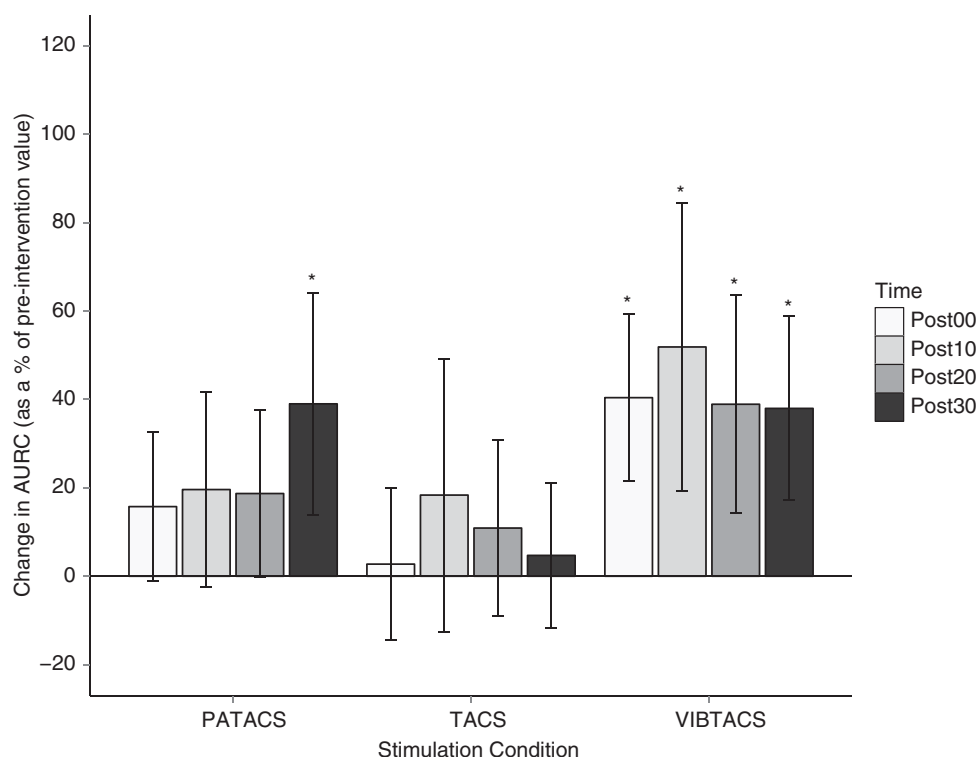


Figure 7. For Experiment 5, in which the nature of the afferent stimulation was varied, the AURC for each post-intervention time point is expressed as the (percentage) change relative to the value obtained prior to the intervention

All values are the mean of 12 participants. The error bars are the corresponding 95% confidence intervals calculated across participants. Values recorded following the intervention that differed reliably ($P < 0.05$) from those obtained prior to the intervention are represented by an asterisk above the error bar.

STDP-orientated accounts, in all cases the delay of the magnetic stimulus with respect to the preceding peripheral shock was 25 ms. It might be argued therefore that in these protocols also, the *effective* ISI was within the range of timing intervals associated with STDP.

In the present study, however, the application of a range of tACS frequencies (80, 140 and 250 Hz), in conjunction with the use of both discrete (i.e. trains of electrical pulses) and continuous (80 Hz tendon vibration) methods of generating peripheral afference, precludes the possibility that the phenomenon was attributable to either a specific order or timing of the stimulus-generated cortical events. In thus revealing that associative effects are expressed when the timing (or order) of the contributory elements is not precisely circumscribed, these findings suggest that multiple cellular pathways may mediate the LTP-type response typically ascribed to PAS.

With regard to the assumption that the associative nature of the stimulation protocol was instrumental in promoting the observed increases in corticospinal excitability, it has been reported on many previous occasions that peripheral nerve stimulation of the type used here (1 ms duration shocks applied at 10 Hz) induces elevations of CSE only when it is applied for extended (≥ 2 h) periods, using high duty cycles (e.g.

50% – 500 ms on; 500 ms off) (Ridding *et al.* 2000, 2001; Charlton, 2003). Similarly, Steyvers *et al.* (2003) and Forner-Cordero *et al.* (2008) have shown that continuous 80 Hz vibration of the FCR muscle tendon applied for 60 min is insufficient to induce either acute or chronic changes in the excitability of corticospinal projections to FCR. In the present study we also demonstrated that administration of the PNS alone was insufficient to bring about changes in corticospinal excitability. Similarly, bursts of tACS (2 mA; 140 Hz; 500 ms duration) – when applied in isolation over a period of 30 min – failed to alter CSE. Sham stimulation was likewise ineffective. It seems reasonable to conclude therefore that the critical factor was the repeated association of tACS with peripheral afferent stimulation.

Recent developments in modelling current density suggest that tACS at the intensities utilised here is capable of altering the membrane potentials of neurons in M1. Neuling *et al.* (2012) demonstrated that superficial areas of grey matter that protrude into the cerebrospinal fluid (a characteristic typical of the M1 representations of upper limb muscles) are likely to be subject to the highest current densities. When 1 mA current is applied, these can reach 0.1 A m^{-2} , and generate electrical fields of up to $417 \text{ } \mu\text{V mm}^{-1}$. In the majority of conditions

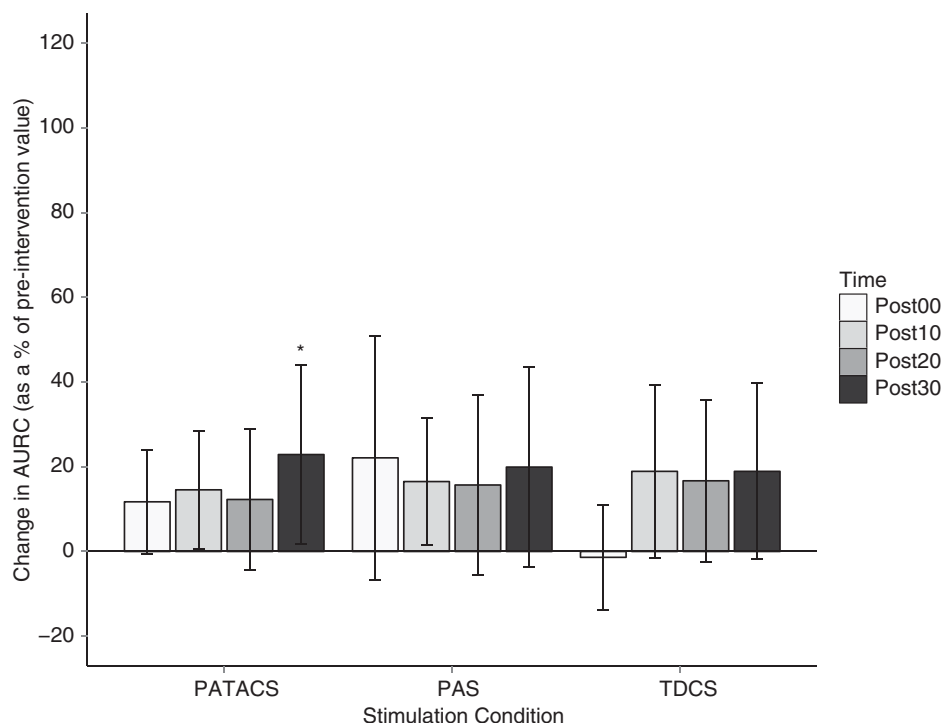


Figure 8. For Experiment 6, in which the effects of PATACS were compared with PAS and tDCS, the AURC for each post-intervention time point is expressed as the (percentage) change relative to the value obtained prior to the intervention

All values are the mean of 12 participants. The error bars are the corresponding 95% confidence intervals calculated across participants. Values recorded following the intervention that differed reliably ($P < 0.05$) from those obtained prior to the intervention are represented by an asterisk above the error bar.

employed in the present study, currents of at least 2 mA were used. These are likely to have been sufficient to provide current densities well in excess of the minimum ($140 \mu\text{V mm}^{-1}$) thought necessary to alter the polarisation of neural membranes (Francis *et al.* 2003). Nonetheless, as such levels of tACS (or indeed tDCS) are not known to generate action potentials in descending corticospinal neurons, it might be supposed that the associative effects were mediated at the level of the cerebral cortex (i.e. rather than at the level of the spinal cord).

It has been hypothesised that direct current stimulation (tDCS) has the potential to bias (i.e. depolarise or hyperpolarise) the membrane potential of cortical neurons (Nitsche & Paulus, 2000). Due to the oscillating polarity of the applied current, it is unlikely that the effects of tACS can be accommodated in these terms. It is currently believed possible, however, that at least in relation to the endogenous frequencies registered traditionally by EEG, tACS is capable of entraining oscillatory activity in the cortex (Antal *et al.* 2008; Antal & Paulus, 2013; Herrmann *et al.* 2013; Helfrich *et al.* 2014). The literature concerning the use of high frequency (i.e. ≥ 80 Hz) tACS to influence intrinsic cortical rhythms is less extensive, although ripple range stimulation (Moliadze *et al.* 2010) and high frequency random noise stimulation (Terney

et al. 2008) have both been used to increase corticospinal excitability. Moliadze *et al.* (2010) speculated that the effect of 140 Hz stimulation in particular could be due to an interaction with sharp wave ripple complexes – short bursts high frequency oscillatory activity thought to be important in memory consolidation (Logothetis *et al.* 2012). The conjecture that ripple range tACS is uniquely influential was not supported by the present results.

It might also be noted in this context that the impact of extended (i.e. 10 min) 140 Hz tACS (potentiating *vs.* inhibiting) is reportedly sensitive to the intensity of stimulation that is applied (Moliadze *et al.* 2012). Specifically, a reduction in corticospinal excitability was demonstrated for 0.4 mA tACS, whereas (as per Moliadze *et al.* 2010) an increase was obtained if 1 mA current was used. Due to variations in the size of electrode placed over the target M1 (25 cm^2 in the present study; 16 cm^2 in the Moliadze *et al.* studies), the current densities resulting from 1 mA stimulation are not equivalent (0.04 *vs.* 0.06 mA cm^{-2}). Nonetheless, at least within the range examined, variations in the level of applied tACS current, and thus in the current density (0.04 mA cm^{-2} at 1 mA, 0.08 mA cm^{-2} at 2 mA and 0.12 mA cm^{-2} at 3 mA), did not reliably influence the magnitude of the effects observed in the present study.

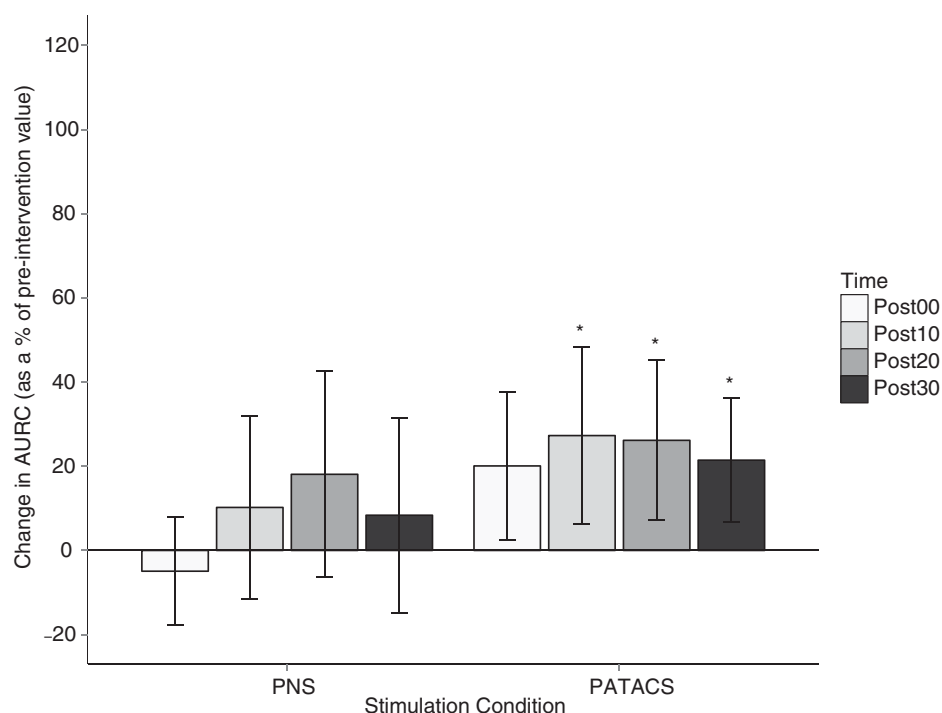


Figure 9. For Experiment 7, in which the effects of PATACS were compared with PNS alone, the AURC for each post-intervention time point is expressed as the (percentage) change relative to the value obtained prior to the intervention

All values are the mean of 17 participants. The error bars are the corresponding 95% confidence intervals calculated across participants. Values recorded following the intervention that differed reliably ($P < 0.05$) from those obtained prior to the intervention are represented by an asterisk above the error bar.

In experiment 1, 80, 140 and 250 Hz tACS – when paired with PNS – all brought about elevated levels of CSE. There remains the possibility that each of these frequencies was responsible for entrainment of a lower endogenous oscillatory frequency through sub-harmonic resonance. Bursts of high frequency (60–90 Hz) gamma oscillations have been ascribed a facilitating role in movement initiation (Cheyne, 2013). Half harmonic resonance at 140 Hz stimulation, quarter harmonic resonance at 250 Hz and first harmonic resonance at 80 Hz could match this high frequency gamma range. In principle, the timing of the peripheral nerve shocks relative to the peaks and troughs of the AC stimulation cycle may also have a determining influence on the effects that are induced.

Whether achieved by high intensity of individual shocks, and/or by elevated frequencies or periods of delivery, increases in the excitability of corticospinal projections from M1 can in some circumstances be induced by afferent stimulation alone (e.g. Ridding *et al.* 2000; Khaslavskaya *et al.* 2002; McKay *et al.* 2002; Knash *et al.* 2003; Chipchase *et al.* 2011; Schabrun *et al.* 2012 – see also Luft *et al.* 2002). The related point that may be made concerning PAS is that, in addition to the relative timing of its delivery in relation to TMS, the intensity of afferent stimulation may play an instrumental role in determining the magnitude of the effects that are induced. Furthermore, these considerations serve to highlight the possibility that the impact of the cortical stimulation delivered over M1 is to augment the weak effects that arise from the peripheral stimulation.

As a corollary of this line of reasoning, dose-dependency might be anticipated. In this regard, we observed that epochs of paired stimulation (onsets staggered by 25 ms) spanning 175 ms were ineffective, whereas 375 and 875 ms periods of simultaneous PNS and tACS gave rise to reliable increases in CSE. When these three conditions were compared directly, however, no statistically reliable differences were apparent. As such, there is presently no evidence that the magnitude of the PATACS-induced effect is dose dependent.

An alternative proposition is that the efficacy, rather than simply the dose, of afferent stimulation is an important determining influence in relation to the effects of associative stimulation. There are differences in the manner in which the corollaries of vibratory and electrical afferent stimuli exert an influence upon circuits within M1. The N20 component of the somatosensory evoked potential, measured by EEG in response to electrical nerve stimulation, is dominated by cutaneous input (Kunesch *et al.* 1995). The origin of the associated N20 response is thought to be a deep tangential generator in area 3b (e.g. Desmedt & Ozaki, 1991; McLaughlin & Kelly, 1993), which has sparse if any connections with M1 (Burton & Fabri, 1995). In contrast, the source generator for cortical potentials invoked by muscle spindle afference

(e.g. in response to tendon vibration) is principally area 3a (Mackinnon *et al.* 2000). As this area has extensive direct projections onto pyramidal and multipolar neurons in deep (V and VI) layers of M1 (Porter *et al.* 1990), it has been highlighted previously that, in the context of associative stimulation protocols, muscle spindle afference – such as that generated by tendon vibration – may represent the most efficacious source of peripheral input (Carson & Kennedy, 2013). Although it was the case that when paired with tACS, muscle tendon vibration induced more reliable elevations of CSE than electrical nerve stimulation, when contrasted directly the PATACS and VIBTACS interventions were not differentiated. The present study therefore failed to provide a basis upon which to conclude that the magnitude of the associative effect is instrumentally related to the modality of afferent stimulation.

Miniussi *et al.* (2013) have proposed that some forms of non-invasive brain stimulation (NIBS) exert their effects through stochastic resonance. In a psychophysical context, it is believed that stochastic noise acts as a pedestal sufficient to raise the effective level of the relevant stimulus above the detection threshold. When delivered in isolation, the forms of afferent stimulation applied in the present study are not known to induce sustained changes in corticospinal excitability. In circumstances in which high frequency tACS is applied concurrently, it is possible that the activity in M1 circuits that receive inputs from sensory areas may be rendered sufficient to alter the state of corticospinal neurons engaged (subsequently) by TMS. In relation to this conjecture, a number of caveats necessarily apply. The stochastic resonance model of NIBS was developed: (i) to explain effects reported during ‘online’ stimulation, as opposed to the ‘offline’ aftereffects obtained here; (ii) in relation to signal detection rather than motor systems plasticity; and (iii) to account for the addition of ‘noise’, rather than a regularly oscillating stimulus. There is no indication in any of the *in vitro* or *in vivo* research reported to date (Reato *et al.* 2013) that tACS applied over the scalp at a fixed frequency translates into random (i.e. broad spectrum) noise in the cortex.

The effects of all forms of non-invasive brain stimulation vary markedly across individuals (Cheeran *et al.* 2008; Ridding & Ziemann, 2010; Hamada *et al.* 2013). In our experiment 6, tDCS and PAS failed to yield reliable increases in corticospinal excitability, despite the use of protocols that are ostensibly effective (for reviews see Nitsche *et al.* 2008; Carson & Kennedy, 2013). In the same group of individuals, PATACS generated an elevation in CSE. Nonetheless, when compared directly the three conditions were undifferentiated. On the other hand, in experiment 3 increases in CSE occurred after 1000 ms (2 mA, 140 Hz) PATACS but not after 500 ms (2 mA, 140 Hz) PATACS – a variant that increased corticospinal

excitability in four other groups of participants. In this context, the value of large samples is clearly revealed by the outcomes of the pooled analysis, which comprised the 64 participants (drawn from five experiments) who were exposed to the same PATACS protocol (i.e. PNS paired with 140 Hz, 2 mA tACS of 500 ms duration). Beyond the increased statistical power that is accrued, the use of larger samples permits the derivation of confidence intervals for the associated effect size estimates. These impose reasonable bounds on the interpretations that may be derived (Smithson, 2001). It follows that in the present instance, given same-sized samples under identical conditions, we should expect that in 95 of 100 repetitions the population value of the effect size for the Pre vs. Post30 comparison (following this form of PATACS) will range between medium ($f = 0.27$) and large ($f = 0.48$).

It might be instructive to further manipulate parameters of the PATACS intervention. The three stimulation frequencies examined herein elevated CSE. Thus, it is as yet unclear whether high frequency entrainment is taking place, or whether stimulation using (i) frequencies of lower than 80 Hz, (ii) frequencies higher than 250 Hz or (iii) high frequency random noise stimulation (tRNS, Terney *et al.* 2008) would be equally effective. Similarly, with respect to the currents that were applied, we did not encounter either a floor or a ceiling effect. It is possible that intensities lower than 1 mA may remain effective (although there are doubts about the efficacy of tACS below this level; see Moliadze *et al.* 2012). In consideration of safety and comfort it is unlikely that current levels will be routinely extended beyond 3 mA. Periods of paired stimulation much shorter than 500 ms may not be effective. There is also a possibility that periods extending beyond 1 s may further accentuate the induced effects. We observed a largely monotonic increase in corticospinal excitability over the 35 min interval following stimulation. While the origin of this pattern of response is unclear, it was reliable, being replicated in each PATACS condition. The extent to which it is sustained thereafter awaits further exploration.

The principal significance of the present study lies in the demonstration that associative effects are expressed when the timing of the peripheral and cortical events is not precisely circumscribed. One interpretation of these findings is that an account of the mechanisms underlying associative plasticity of the human motor system that emphasises only STDP is unlikely to be complete. The results suggest instead that multiple cellular pathways contribute to the LTP-type response that is engendered by associative stimulation protocols.

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Additional information

Competing interests

The authors declare that they have no competing interests in relation to the work that is reported.

Author contributions

Both authors contributed substantially to the conception and design of the study, and to the analysis and interpretation of the data. The authors together wrote the article, jointly contributed the intellectual content and gave approval to the final version of the article to be published.

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